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Survival of Human Immunodeficiency Virus in Suspension and Dried onto Surfaces

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Cell-free and cell-associated human immunodeficiency virus cultures suspended in 10% serum remained infectious for several weeks at room temperature. The stability was further increased when cell-associated virus was suspended in neat serum. When dried onto a glass coverslip, virus remained infectious for several days, although cell-associated virus lost infectivity more rapidly than cell-free virus.

Many parameters influence the capacity of a virus to survive in the environment, including the concentration of virus, the temperature, the humidity, and the nature of the surrounding medium. The rate at which infectivity is lost is an important determinant of the potential risk of transmission.

The pitfalls which may occur when using model systems to describe the survival characteristics of a virus have been reported previously (29). A human rotavirus isolate and a simian rotavirus had equivalent stabilities under certain conditions but not others. Similarly, a comparison of these two rotaviruses with poliovirus showed that the latter was more labile in some conditions but more stable in others.

Early studies on the survival of human immunodeficiency virus (HIV) are difficult to interpret because of differences in methodology. The first report, by Barré-Sinoussi et al. (4), gave no details of the suspension medium and the presence of virus was monitored by the use of reverse transcriptase, an indirect and relatively insensitive assay. Resnick et al. (27) suspended virus in medium supplemented with 50% human plasma. Determination of infectivity was by cell culture, a direct measure of infectious virus, as well as by assay of viral reverse transcriptase. Despite these differences and the possible underestimation of infectious virus, both studies reported survival for several days.

In our study, we used the more sensitive techniques now available to examine the survival of virus in liquid suspension at room temperature and dried on glass. Initially, the virus was suspended in standard tissue culture medium containing 10% serum, but to assess the effect of the high protein levels, which is more representative of the in vivo situation, cell-associated virus (CAV) suspended in neat serum was also tested.

The RF strain of HIV was maintained by continuous culture in MT-4 cells suspended in complete medium (RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum [FCS], 5 mmol *N*-2-hydroxyethylpiperazine-*N'*-2-ethansulfonic acid [HEPES] liter^{-1}, and 50 mg of gentamicin liter^{-1}). Stock cell-free virus (CFV) was obtained by removal of the supernatant after centrifugation of the culture at 830 × *g* for 15 min. The resulting pellet was washed twice in phosphate-buffered saline and was resuspended in test medium to provide CAV. The standard test medium was complete medium (CM) containing 10% FCS. Neat (100%) FCS was used to determine the effect of an increased protein load on CAV.

Survival of virus in suspension. Aliquots (1 ml) of virus (CFV or CAV) were pipetted into 2-ml plastic microtubes, left on the laboratory bench for the required time, and titrated in quadruplicate into MT-4 cells in 24-well tissue culture plates. The plates were incubated at 37°C in 5% CO_2. The room temperature of the laboratory during the test period was in the range of 20 to 28°C.

Survival of virus dried onto a surface. Aliquots (100 μl) of 10-fold virus dilutions (CFV or CAV) were pipetted onto sterile 13-mm-diameter glass coverslips and were allowed to dry rapidly over silica gel in a class I safety cabinet. The drying time was approximately 2.5 h. Dried coverslips were transferred aseptically to 24-well tissue culture plates and were left on the laboratory bench for the test period, after which a 1-ml suspension of MT-4 cells was added to each well. The plates were incubated at 37°C in 5% CO_2. Loss of infectivity because of drying alone was measured by a comparison of the titer of the stock virus suspension with the titer of virus from dried virus dilutions sampled immediately after drying. The room temperature of the laboratory during the test period was in the range of 20 to 28°C. Each experiment was done once, with samples taken in quadruplicate. Preliminary studies were also done with blood. Fresh human blood from healthy volunteers was collected into tubes containing the anticoagulant EDTA, and the tubes were mixed by inverting them a minimum of six times. Tenfold dilutions of CFV (100 μl) were added to 900 μl of blood, and the protocol was continued as described above.

The presence of infectious virus was determined by examining viral cultures every 3 to 4 days for a cytopathic effect. The 50% tissue culture infective dose ($TCID_{50}$) per milliliter was calculated by the method of Kärber (17). Replicating virus was measured by determining the level of p24 core antigen every 7 to 10 days by using the DuPont enzyme-linked immunosorbent assay (ELISA) kit. Growth of virus would be indicated by antigen levels remaining high or rising for the first few weeks of repeated subculture, whereas nonreplicating virus would be gradually diluted out, resulting in a fall in antigen titer.

The results presented in Fig. 1 show that a reduction in viral titer of nearly 8 log $TCID_{50}$s of CFV per ml and over 6 log $TCID_{50}$s of CAV per ml took between 4 and 5 weeks (28 and 35 days) when virus was suspended in 10% serum. When

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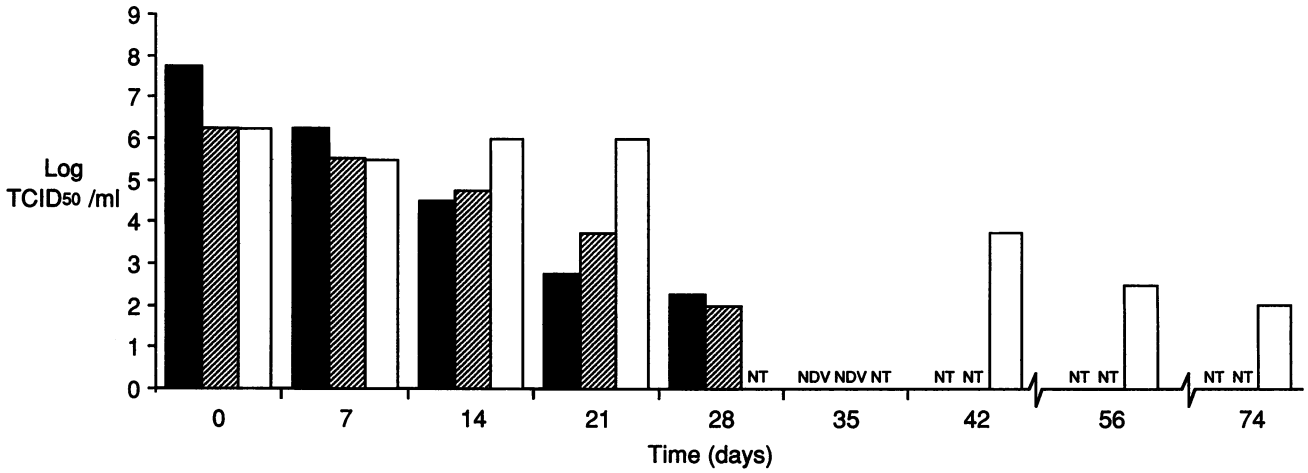

FIG. 1. Aliquots (1 ml) of CFV in 10% serum (■), CAV in 10% serum (▨), or CAV in 100% serum (□) were pipetted into plastic tubes, left on the laboratory bench for the test period, and subsequently titrated into cells. Infectivity and growth of virus were determined by measuring $TCID_{50}$s and p24 antigen levels, respectively. NDV, no detectable virus; NT, not tested.

the same titer of CAV was suspended in neat (100%) serum, 2 log $TCID_{50}$s of virus per ml were still present after 10 weeks, indicating a loss in titer of over 4 log $TCID_{50}$s/ml. These results may be expressed as a rate of inactivation, that is, the time taken for a 1-log-unit loss of infectivity, which is known as the *D* value (Table 1). The *D* values for CFV and CAV in a suspension of 10% serum were 5 and 6 days (122 and 146 h), respectively. At high protein levels (CAV in neat serum), this increased to approximately 13 days (308 h), demonstrating the protective effect of protein.

CAV dried onto glass coverslips survived less well than CFV dried under similar conditions (Fig. 2). Loss of infectivity of over 6-log $TCID_{50}$s of CAV per ml in CM containing 10% serum took about 6 days, giving a *D* value of less than 1 day (Table 1). The titer of CFV was only slightly reduced after 1 week, the longest time tested. If a linear decrease in viral titer is assumed, the results obtained indicate a *D* value of greater than 70.2 h. The preliminary studies used to determine the survival of CFV in dried blood proved to be problematic because of the brittle nature of the thin layer of blood on the glass coverslips and the subsequent loss of small fragments of blood, leading to a possible underestimation of the amount of virus present. Nevertheless, those studies indicated that the rate of inactivation was probably no less than that of CFV in 10% serum (data not shown). Quantitative experiments were not possible.

Qualitative results of the survival times for virus dried in CM at a range of initial titers from 0.5 to 6.5 log $TCID_{50}$s/ml are shown in Tables 2 and 3. CAV at a titer of 4.5 log $TCID_{50}$s/ml took between 4 and 5 days to be inactivated, whereas at a lower titer of 1.5 log $TCID_{50}$s/ml it took less than 3 days. In contrast, 1.5 log $TCID_{50}$s of CFV per ml could still be recovered after 6 days. The loss of titer because of drying the virus onto the carrier was never more than 1 log $TCID_{50}$/ml.

The capacity of HIV to survive at room temperature in suspension and in the dried state was similar to that described by other workers under a variety of environmental conditions. In one of the earliest publications (4), it was reported that HIV survived drying at room temperature and retained activity for 7 days. However, the viral state (CAV or CFV) was not made clear and the survival data were based on measurement of reverse transcriptase activity. Later reports have confirmed that the survival of HIV for several days is similar to that of other enveloped viruses (5,

TABLE 1. Survival of virus at room temperature

Virus	Medium	*D* value (h)[a]
In suspension		
CFV	CM	122
CAV	CM	146
CAV	FCS	308
Surface dried		
CAV	CM	17.5
CFV	CM	>70.2

[a] The *D* value is the time taken for a loss in infectivity of 1 log $TCID_{50}$/ml.

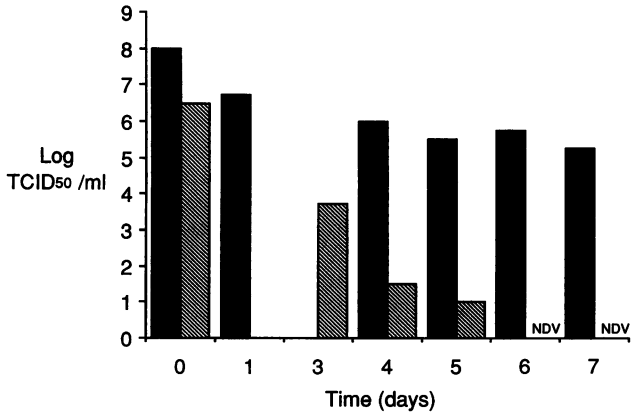

FIG. 2. Aliquots (100 μl) of 10-fold dilutions of CFV (■) or CAV (▨) in 10% serum were dried onto glass coverslips, transferred to 24-well plates, and left on the laboratory bench for the test period. $TCID_{50}$s were determined after culturing of virus with cells for 21 days. NDV, no detectable virus.

TABLE 2. Survival of CAV in CM (10% serum) dried onto glass coverslips

Initial log $TCID_{50}$	Presence or absence of virus after the following drying times (days):				
	0	3	4	5	6
5.5	+	+	+	+	−
4.5	+	+	+	−	−
3.5	+	+	−	−	−
2.5	+	+	−	−	−
1.5	+	−	−	−	−
0.5	−	−	−	−	−

13, 18, 27). The survival characteristics of HIV have been attributed, in part, to its lipid envelope, which resists drying and protects internal viral constituents from the deleterious effects of dehydration (10).

Our results, which are in agreement with those of Resnick et al. (27), show that CFV appears to possess a greater capacity for survival than CAV. This can be attributed to the barrier effect of dried cellular material, which may protect the virus from adverse environmental conditions and may also prevent the replication or release of surviving virus. It is not possible to discriminate between the genuine loss of viral infectivity and the inability of the cell recovery system to retrieve surviving virus from within the cellular debris.

The results of our work and those of other investigators must be interpreted with caution because virus survival can be affected by many factors. Moreover, there may be unknown additional stabilizing or destabilizing effects of the conditions to which the virus may be exposed. Differences in media and pH may in part explain the discrepancy in results obtained between some workers. Tjøtta et al. (30) demonstrated the effect of pH on the rate of survival of HIV at room temperature. At the optimal pH (7.0 to 7.1), survival of virus in suspension was comparable to that of virus dried onto a glass surface. Their results were expressed as a half-life of between 30 and 35 h, equivalent to *D* values of between 4 and 5 days. This is similar to our *D* value for CFV in a suspension of 10% serum (5 days), but differs considerably from our *D* values for dried CAV (17.5 h) or CAV in neat serum (13 days). We did not measure the pH in our studies.

Another factor of potential importance is that of temperature. HIV survival for several days has been reported within cadavers stored refrigerated or nonrefrigerated (2, 3, 14, 23, 26). Environmental survival of viruses is particularly affected by relative humidity (20, 28), which can vary considerably. We did not control for temperature, humidity, or sunlight, because we believed that leaving the samples on our laboratory bench was representative of the in vivo situation in a typical laboratory in a temperate climate.

TABLE 3. Survival of CFV in CM (10% serum) dried onto glass coverslips

Initial log $TCID_{50}$	Presence or absence of virus after the following drying times (days):					
	0	1	4	5	6	7
6.5	+	+	+	+	+	+
5.5	+	+	+	+	+	+
4.5	+	+	+	+	+	+
3.5	+	+	+	+	+	+
2.5	+	+	+	+	+	+
1.5	+	+	+	−	+	−
0.5	+	+	−	−	−	−

Other parameters influencing virus survival include the viral strain and the medium in which the virus is grown. Studies involving virus detection by PCR (16) and culture in semen (1) and plasma (24) have indicated that the infectivities of wild-type strains of HIV may vary and differ significantly from those of culture-adapted virus. Moudgil and Daar (22) have shown variability in the infectious decay of HIV in the plasma of infected patients in samples stored at room temperature. Infectious virus was present after 7 days in four of five specimens. Pan et al. (24) described a substantial loss of endogenous infectious virus in plasma specimens left at room temperature for more than 3 h after venipuncture. However, the titer of virus isolates added to normal human plasma remained stable over 24 h, whereas plasma from infected individuals inactivated those virus strains susceptible to neutralization. This appeared to be associated, in part, with the immunoglobulin G fraction of blood and may explain the variations between different isolates and culture-adapted virus.

Despite the many variables which might affect the survival of HIV in the environment, our results can still be extrapolated to the in vivo situation. A maximum titer of HIV of 4 log units per ml has been recorded in the blood of patients with AIDS (15, 21) and in the blood of individuals with primary infection in which there were often transient high levels of viremia (9). Under our test conditions, loss of infectivity of such high titers would not occur until 4 to 8 weeks. Despite this long survival time, we know of no evidence that HIV is capable of being transmitted via contaminated fomites, although the possibility of that risk cannot be excluded. Virus present in many clinical specimens or spillages may lose infectivity within a few days. Most specimens contain only low concentrations of virus, and HIV is not known to be transmitted via any route other than by the already well documented ones of sexual contact, parenteral inoculation, vertical transmission, and transfusion or transplantation of contaminated organs, tissues, and blood products (6–8, 11, 12, 19, 25, 31). We would nevertheless emphasize that suitable precautions must be taken at all times when dealing with infectious or potentially infectious material.

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